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**Applicant(s)**

Anne Y. Fu, Hacienda Heights, CA;
Stephen Quake, San Marino, CA;

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**Title**

Microfluidic devices and methods of use

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# PATENT APPLICATION

## Microfluidic Devices and Methods of Use

Inventor(s):

Ann Y. Fu

Stephen Quake

Assignee:

Mycometrix
213 E. Grand Avenue
South San Francisco, CA 94080

Entity: Small entity

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: 303-571-4000

# Microfluidic Devices and Methods of Use

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

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## DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention is directed to microfluidic devices comprising pumps, valves, and fluid oscillation dampers. In this respect, the microfluidic devices of the present invention is similar to that described in Unger et al. *Science*, **2000**, *288*, 113-116, which is incorporated herein by reference in its entirety. However, microfluidic devices of the present invention further comprise a damper.

The advantages of microfluidic devices of the present invention include reduced fluid oscillation within a flow channel which reduces potential variability in detection means. As the fluid is pushed through the flow channel by the pumps, there is a tendency for the fluid to oscillate, i.e., the fluid is pushed through the flow channel in a sinusoidal wave-like fashion. As this oscillating fluid passes through a detector region, different fluid depth passes through the detector region. And depending on a particular detection means used, this difference in fluid depth can cause a higher "background noise" or an inaccurate reading by the detector. By reducing or eliminating this fluid oscillation, the "background noise" is reduced and a more accurate reading by the detector can be achieved.

Preferred devices are constructed by single and multilayer soft lithography (MLSL) as detailed in commonly assigned U.S. Patent Serial Application No. 09/605,520, filed June 27, 2000, which is incorporated herein by reference in its entirety.

Microfluidic devices of the present invention comprise an integrated pump which can be electronic, magnetic, mechanical, or preferably pneumatic pumps. By using a pneumatic pump, microfluidic devices of the present invention allow more precise control of the fluid flow within the fluid channel. In addition, unlike electro-osmotic driven fluid flow, pneumatic pump allows the flow of fluids in both directions, thereby

allowing reversible sorting of materials, as discussed in greater detail below. Furthermore, a pneumatic pump provides at least 10 times, preferably at least about 20 times, and more preferably at least about 30 times the fluid flow rate capacity compared to the capacity of electro-osmotic fluid flow.

In addition, microfluidic devices of the present invention comprise a damper which reduces or eliminates the fluid oscillation within the fluid channel. The damper can any device which attenuates the fluid oscillation. For example, the damper can simply be a channel which is open to the ambient atmosphere and has a thin elastic membrane between the channel and the fluid flow channel. Preferably, the damper is an encapsulated pocket of fluid medium with a thin elastic membrane above the fluid flow channel. The fluid medium can be a liquid or, preferably, a gas. The damper is generally located above the fluid flow channels with a thin membrane, preferably an elastic membrane, between the fluid flow channel and the damper. Typically, there is at least 1 damper posterior to the pump in the direction of the fluid flow. Preferably, there is at least 2 dampers, more preferably at least 3 dampers and most preferably at least about 5 dampers posterior to the pump.

The width of damper is at least as wide as the width of flow channel that is located below the damper. In this manner, the entire cross-section of the flow channel is covered by the damper to ensure attenuation of fluid oscillation across the entire width of the flow channel. Preferably, the width of damper is at least about 1.1 times the width of flow channel, more preferably at least about 1.3 times the width of the flow channel, and most preferably at least about 1.5 times the width of the flow channel. In this manner, the need for a precise alignment of the damper on top of the flow channel is eliminated.

The damper is separated from the fluid flow channel by a thin membrane. Preferably this thin membrane has sufficient elasticity to deflect "upward" when a fluid having a peak of sinusoidal wave-like passes underneath. In this manner, some of the fluid oscillation energy is absorbed by the damper, thereby reducing the height (i.e., peak) of fluid oscillation. Typically, the thickness of the membrane between the damper and the fluid flow channel depends on a variety of factors including the depth and width of the flow channel, the amount of fluid oscillation produced by the pump and the elasticity (i.e., the material) of the membrane. One of ordinary skill in the art can readily determine the proper membrane thickness to achieve a desired attenuation of fluid oscillation depending on a desired application and materials used.

In one particular embodiment of the present invention, the microfluidic device comprises a T-channel for sorting materials (e.g., cells or large molecules such as peptides, DNA's and other polymers) with fluid flow channel dimensions of about 50 μm x 35 μm (width x depth). The width of pressure channels (i.e., pneumatic pump) and the damper is 100 μm and 80 μm, respectively. The gap between the flow channel and the damper (or the pressure channel) is about 5 to 6 μm. In order to produce such a thin first layer, the MLSL process requires providing a layer of an elastomer (e.g., by spreading) which is typically thinner than most other previously disclosed microfluidic devices. For example, when using GE RTV 615 PDMS silicon rubber, previous microfluidic devices typically used 30:1 ratio of 615A:615B at 2000 rpm spin-coating to fabricate the first (i.e., bottom) layer of the elastomer and 3:1 ratio of A:B for the second elastomer layer. However, it has been found by the present inventors that the silicon rubber does not cure when the ratio of 30:1 is used in fabricating the above described dimensions of fluid flow channels in the first elastomer layer. Moreover, in order to produce a thin first elastomer layer, a higher spin-coating rate was required. For example, without using any diluent, GE RTV 615 PDMS silicon rubber A and B components in the ratio of about 20:1 was required at 8000 rpm to produce the first elastomer layer having about 3.5 μm flow channel depth and about 5-6 μm thickness between the flow channel and the damper (or the pressure channels). When SF-96 diluent was used, spin-coating at about 3000 rpm can be used to achieve a similar dimension first elastomer layer.

During fabrication of a mold, the photoresist is typically etched using a mask, developed and heated. Heating of the developed photoresist reshapes trapezoid-shaped "ridges", which ultimately form the channels, to a smooth rounded ridges and reduces the height of ridges from about 20 μm to about 5 μm. This method, however, does not provide channels having depth of about 3.5 μm. The present inventors have found that this limitation can be overcome by treating the developed photoresist with oxygen plasma (e.g., using SPI Plasma Prep II from SPI Supplies a Division of Structure Probe, Inc., West Chester, PA) and heating the photoresist at a lower heat setting. Unlike previous methods, where a higher heat setting appear to chemically modify the photoresist, the lower heat setting used in the present invention does not chemically alter the photoresist.

Microfluidic devices of the present invention can be used in a variety of applications such as sorting cells as disclosed in commonly assigned U.S. Patent

Application Serial No. 09/325,667 and the corresponding published PCT Application No. US99/13050, and sorting DNA's as disclosed in commonly assigned U.S. Patent Application Serial No. 09/499,943, all of which are incorporated herein by reference in their entirety.

The actual dimensions of a particular microfluidic device depend on its application. For example, for sorting bacteria which typically have cell size of about 1 μm, the width of fluid flow channel is generally in the range of from about 5 μm to about 50 μm and the depth of at least about 5 μm. For sorting mammalian cells which have typically have cell size of about 30 μm, the width of fluid flow channel is generally in the range of from about 40 μm to about 60 μm and the depth of at least about 40 μm. For DNA sorting, the dimensions of fluid flow channels can be significantly less.

One particular embodiment of the present invention is shown in Figures 1A and 1B, where Figure 1A is a schematic drawing of the microfluidic device shown in Figure 1B. In this embodiment, the microfluidic device comprises an injection pool **22**, where a fluid containing a material can be introduced. The fluid is then pumped through the fluid flow channel **34** via a pneumatic pump **10** which comprises three pressure channels. By alternately pressurizing the three pressure channels, one can pump the fluid through the fluid flow channel **34** in a similar fashion to a peristaltic pump. The fluid exiting the pump oscillates due to actions of the pump. The fluid oscillation amplitude is attenuated by dampers **14** which is located above the flow channel **34**, behind the pump **10** and before a detector (not shown). Initially, the collection valve **18A** is closed and the waste valve **18B** is open to allow the fluid to flow from the injection pool **22** through the T-junction **38** and into the waste pool **30**. When a desired material is detected by the detector (not shown), the waste valve **18B** is closed and the collection valve **18A** is opened to allow the material to be collected in the collection pool **26**. The valves **18A** and **18B** are interconnected to the detector through a computer or other automated system to allow opening and closing of appropriate valves depending on whether a desired material is detected or not.

One such application for the microfluidic device described above is in a reverse sorting of a material (e.g., beads, DNA's, peptides or other polymers, or cells) as shown in Figures 2A and 2B. In the reverse sorting process, the material is allowed to flow towards the waste pool **30** as shown in Figure 2A. When a desired material is detected by a detector (not shown) the pump (not shown) is reversed until the material is

again detected by the detector. At this point, the waste valve **18B** is closed and the collection valve **18A** is opened, as shown in Figure 2B, and the flow of material is again reversed to allow the material to flow into the collection pool **26**. After which the collection valve **18A** is closed and the waste valve **18B** is opened. This entire process is repeated until a desired amount of materials in the injection (or input) pool **22** is "sorted".

In one embodiment of the present invention, *E. Coli* expressing GFP is sorted using the reversible sorting process described above. As shown in Figures 3A and 3B, the cell velocity depends on the frequency of the pump. Thus, the cell velocity reaches a maximum of about 16 mm/sec at about 100 Hz of pump rate. Moreover, as expected, the mean reverse time in Figure 3B, which represents the time interval between detection of *E. Coli* expressing GFP, reversing the pump, and detection of the same *E. Coli*, decreases as the pump frequency is increased.

In another embodiment of the present invention provides sorting materials according to ratio of wavelengths (e.g., from laser induced fluorescence). For example, by measuring two different fluorescence wavelengths (e.g., $\lambda 1$ and $\lambda 2$) and calculating the ratio of $\lambda 1$ and $\lambda 2$, one can determine a variety of information regarding the material, such as the life cycle stage of cells, the stage of evolution of cells, the strength of enzyme-substrate binding, the strength of drug interactions with cells, receptors or enzymes, and other useful biological and non-biological interactions.

Another embodiment of the present invention provides multiple interrogation (i.e., observation or detection) of the same material at different time intervals. For example, by closing on of the valves 18A or 18B in Figure 4C and alternately pumping the fluid to and from the input well **22** at a particular intervals, the material can be made to flow to and from the input well **22** through the detector (not shown). By oscillating this material through the detection window **40**, one can observe the material at different time intervals. For example, a sample can be interrogated at 10 Hz pump frequency as shown in Figure 4A or at 75 Hz pump frequency as shown in Figure 4B. As expected, at a higher pump frequency, the material can be observed at shorter intervals. Such observation of materials at different time has variety of applications including monitoring cell developments, enzyme-substrate interactions, affect of drugs on a given cell or enzyme; measuring half-life of a given material including drugs, compounds, polymers and the like; as well as other biological applications.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter.

WHAT IS CLAIMED IS:

1. A microfluidic device comprising:

a flow channel;

a pump operatively interconnected to said flow channel for moving a fluid in said flow channel; and

a damper operatively interconnected to said flow channel for reducing the fluid oscillation in said flow channel.

2. The microfluidic device of Claim 1, further comprising a flow control valve operatively interconnected to said flow channel for closing and opening said flow channel.

3. The microfluidic device of Claim 2, further comprising a T-junction.

4. The microfluidic device of Claim 3, wherein said T-junction comprise an injection pool, a waste pool and a collection pool interconnected by said flow channel, and wherein flow channel further comprises said flow control valve proximal to said waste pool and said flow control valve proximal to said collection pool, said pump proximal to said injection pool and said damper proximal to said injection pool but posterial to said pump.

5. The microfluidic device of Claim 4 further comprising a plurality of said damper.

6. A method for sorting a material using a microfluidic device of Claim 4.

7. The method of Claim 6, comprising using a reversible sorting process.

8. A method for observing a material at different time intervals using a microfluidic device of Claim 4, wherein said method comprises:

(a) pumping a fluid medium comprising a material away from said injection pool;

(b) detecting said material flowing through said T-junction;

(c) reversing the flow of said material back through said T-junction towards said injection pool and detecting said material; and

(d) optionally repeating said steps (a) to (c).

## ABSTRACT OF THE DISCLOSURE

The present invention provides a microfluidic device comprising pumps, valves, and fluid oscillation dampers.

# Cell Sorter on-Chip

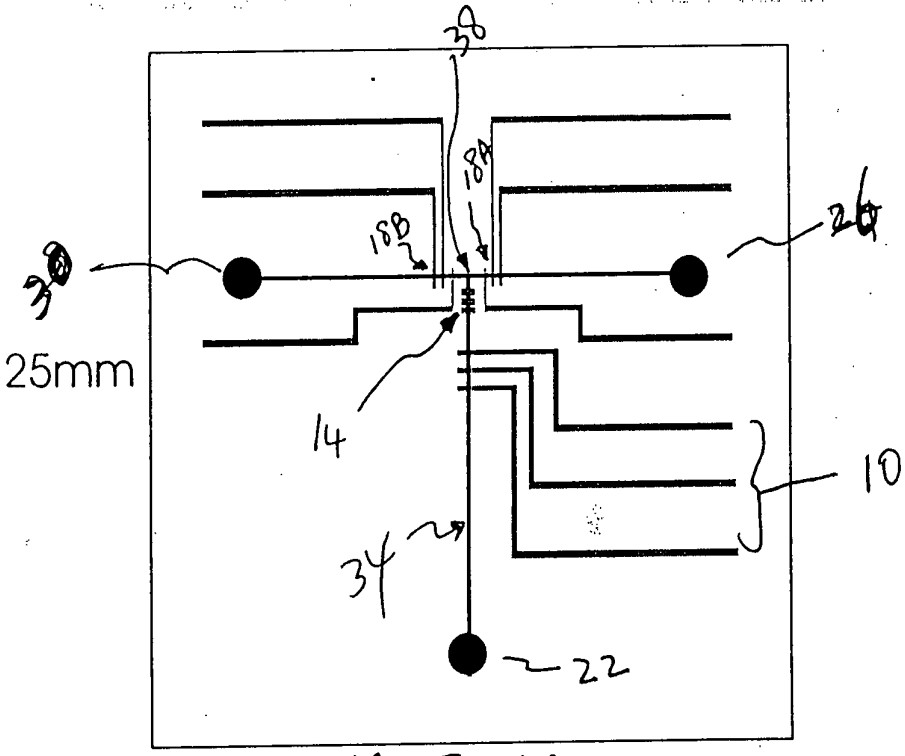



FIGURE 1A

Peristaltic pumps: 100 um
Dampers: 80 um
Switch valves: 30, 50um

T Channel: 50 x 3.5 um
@ T-junction: 5 x 3.5um

interlayer thickness: 6um

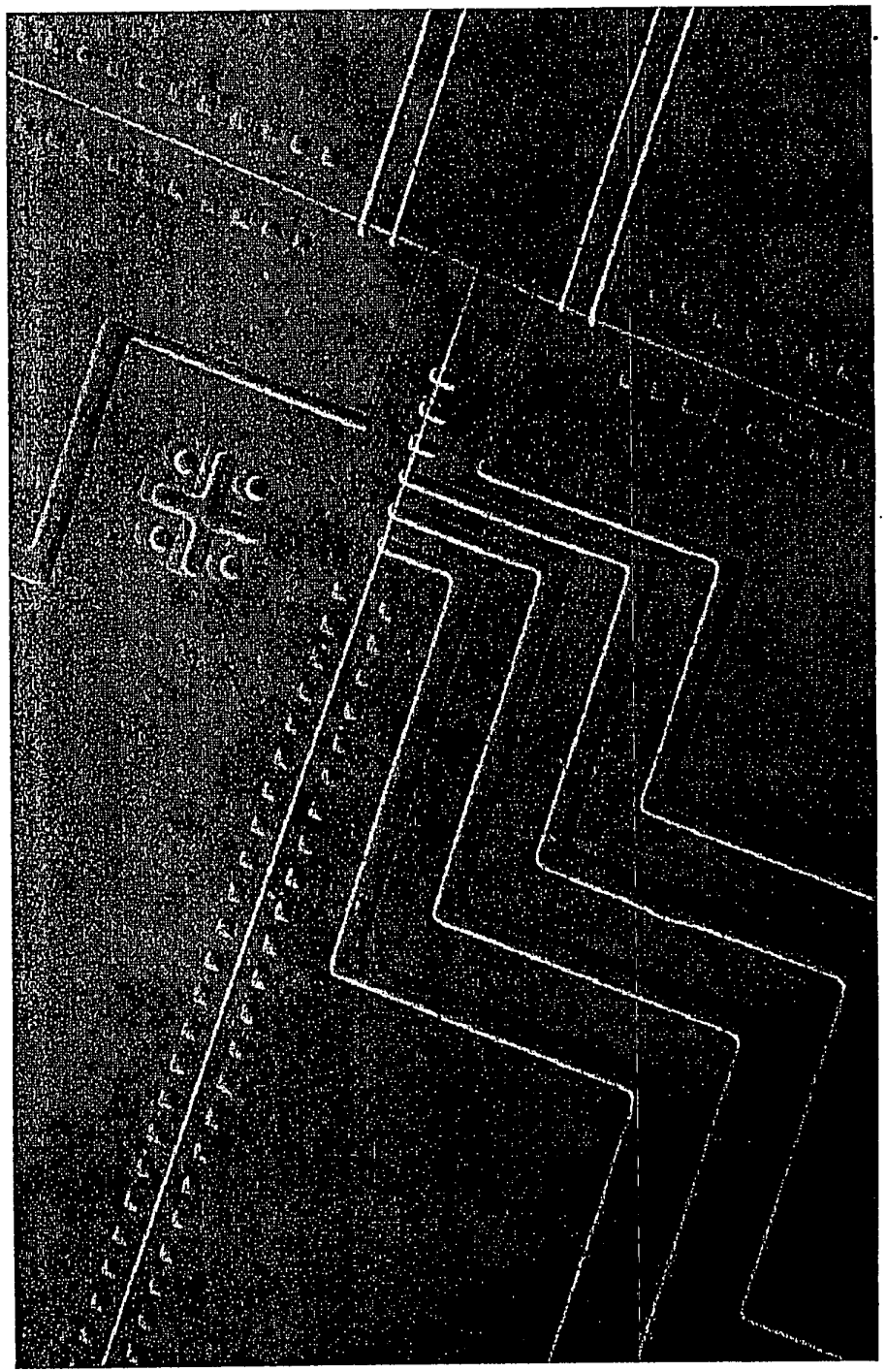


FIGURE 1B

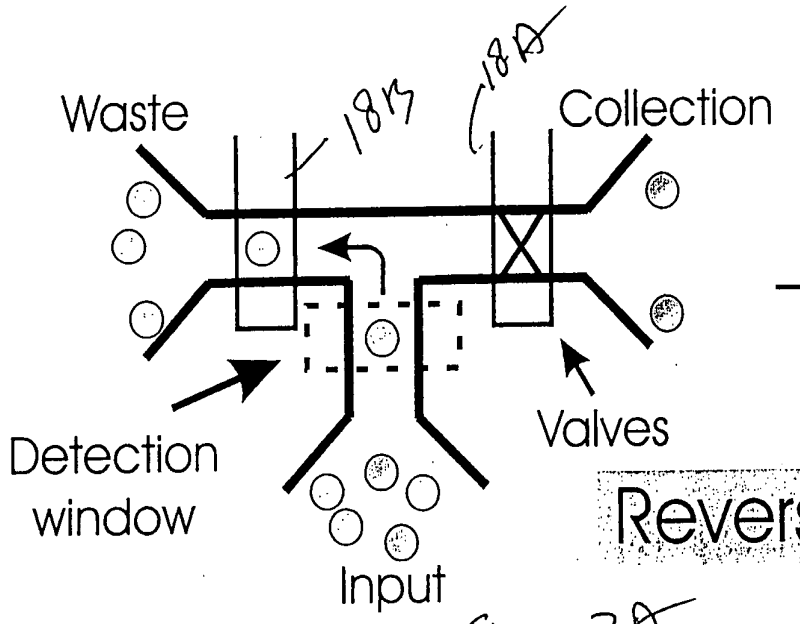



Figure 2A

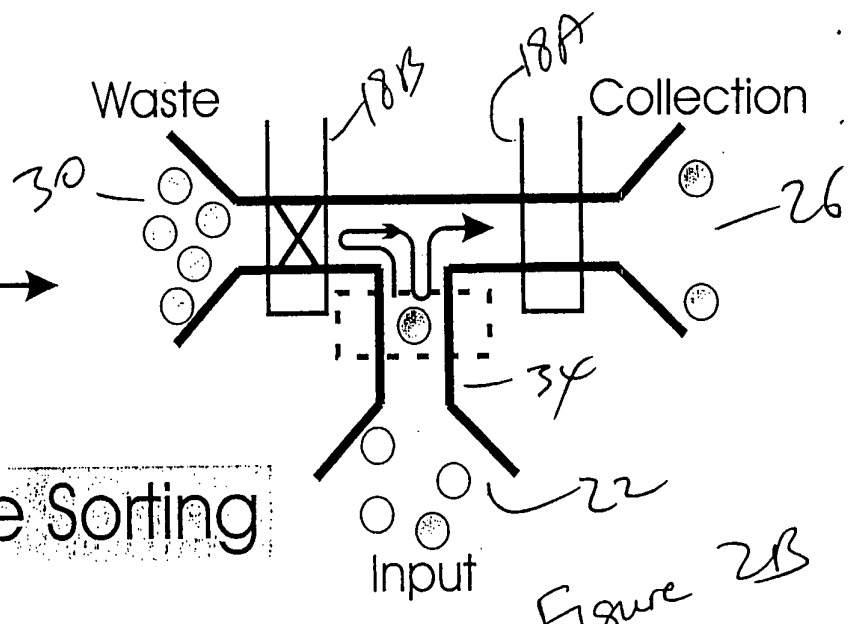
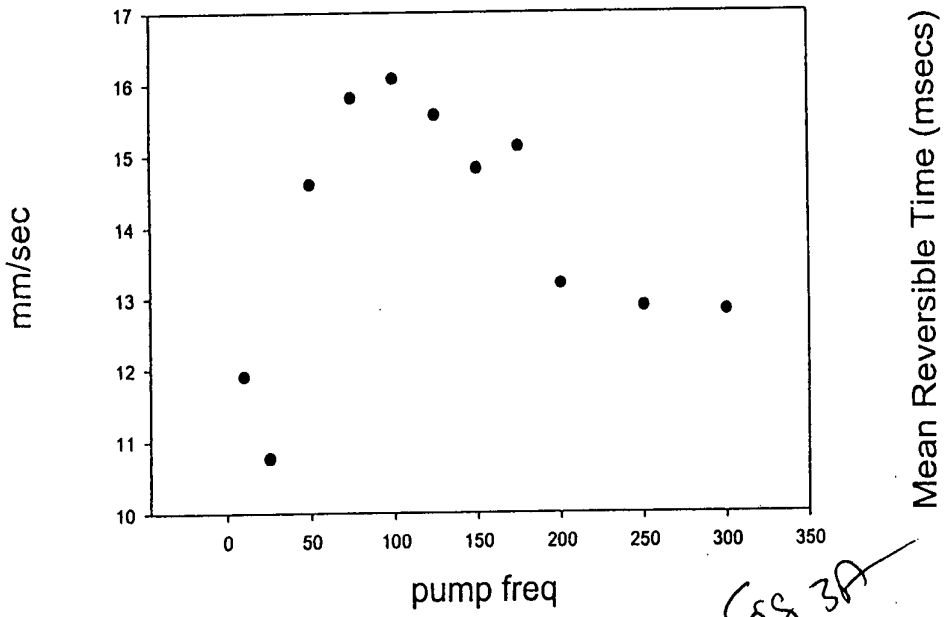



Figure 2B

Reversible Sorting

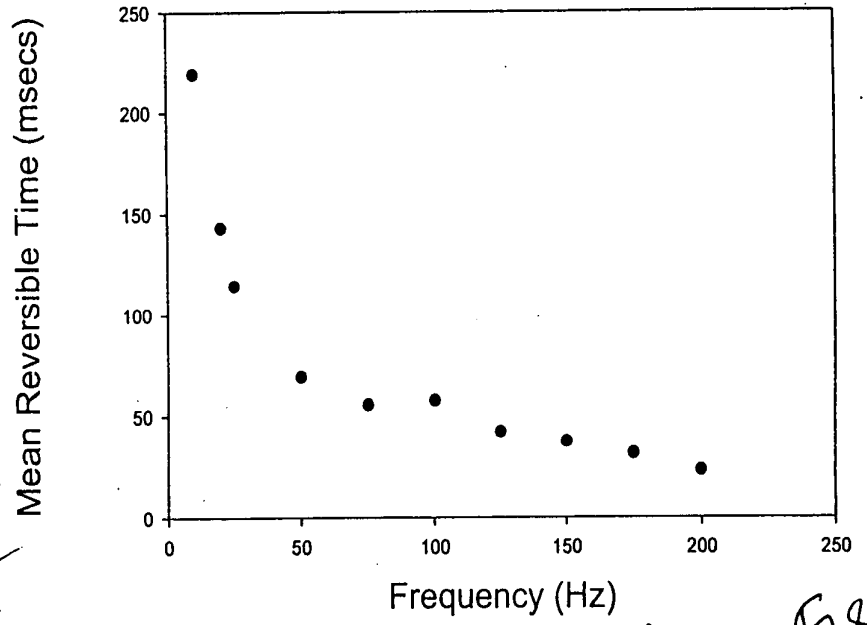
# Characteristics of Sorter

Measured with *E. Coli* cells expressing GFP




Pump Freq vs. Cell Velocity

Fig 3A




Pump Freq vs. Reversible Time

Fig 3B

# Trapping

Multiple interrogation of the same cell at different times!!

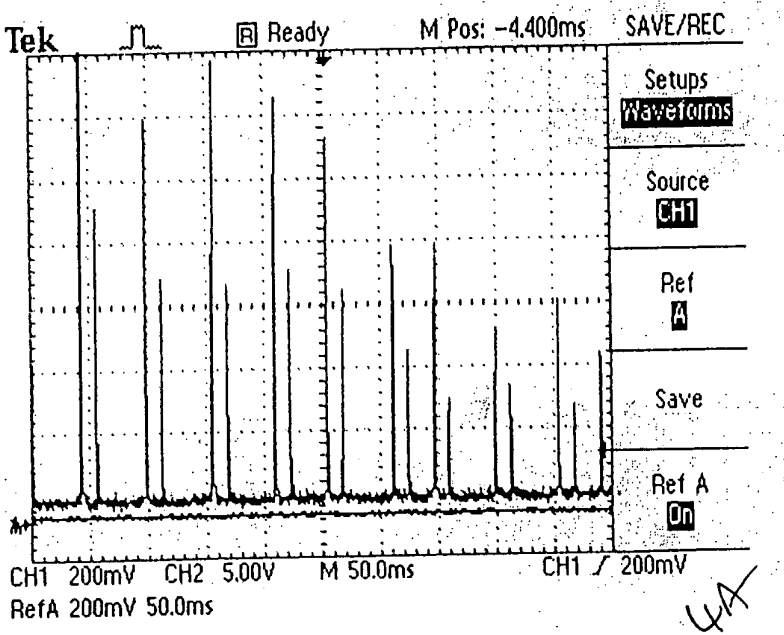



Figure 4A

10 Hz pump frequency

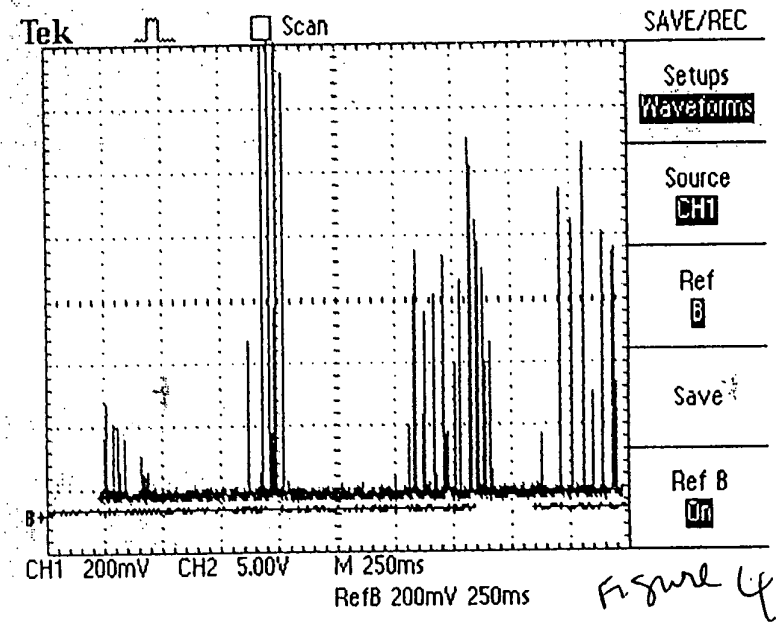



Figure 4B

RAW DATA

75 Hz pump frequency

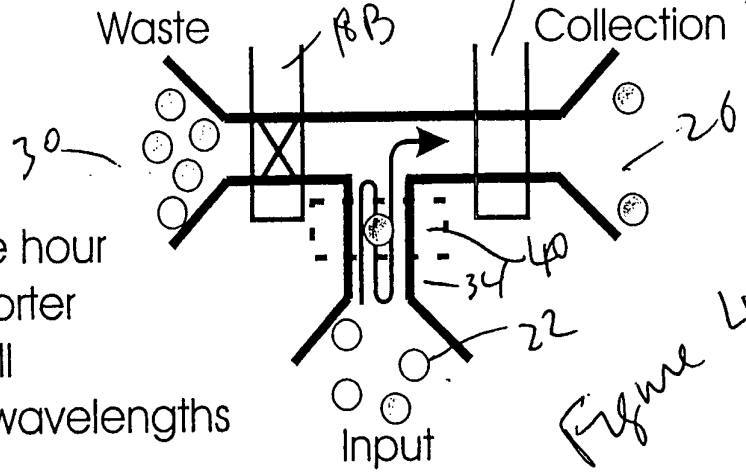



Figure 4A

Figure 4C

# Future Plans

1. Accomplishing reversible sorting of cells $10^6$ in one hour
2. 30 times faster than the original electro-osmotic sorter
3. Study time course measurements of the same cell
4. Sorting mutant GFP libraries according to ratio of wavelengths